BENEFICIAL EFFECT OF CREATININE AND CREATINE ON GROWTH[1]

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(WITH ONE FIGURE)

This paper embodies a series of experiments on the influence of creatinine and creatine on seedling wheat. These experiments were made in an endeavor to throw light on the action of organic manures in soils, and the influence of soil organic matter on productivity. Creatinine has been discovered as a soil constituent in this laboratory by Dr. E. C. SHOREY,[2] and an account of its occurrence and properties will be given elsewhere. This nitrogenous constituent occurs plentifully in animal products, wine, meat, etc., but has recently been found in these laboratories by Dr. M. X. SULLIVAN[3] to be a constituent part of many plants and seeds, and to occur in the medium in which plants have grown. The general methods for studying the effect of creatinine on plants in solution cultures is the same as that employed in connection with the harmful soil constituent, dihydroxystearic acid, previously reported in this journal.[4]

Effect of creatinine on growth

Two sets of cultures, composed of the fertilizer salts calcium acid phosphate, sodium nitrate, and potassium sulphate in varying proportions, used singly and in combinations of two and three, were prepared, the proportions varying in 10 per cent stages, thus making a total of 66 culture solutions according to the plan in the

[1] Published by permission of the Secretary of Agriculture, from the Laboratory of Soil Fertility Investigations.

[2] SHOREY, EDMUND C., The isolation of creatinine from soils. Jour. Amer. Chem. Soc. **34**:99. 1912.

[3] SULLIVAN, M. X., The origin of creatinine in soils. Jour. Amer. Chem. Soc. **33**:2035. 1911.

[4] SCHREINER, O., and SKINNER, J. J., Some effects of a harmful organic soil constituent. BOT. GAZ. **50**:161. 1910; Ratio of phosphate, nitrate, and potassium on absorption and growth. BOT. GAZ. **50**:1. 1910.

papers cited. Young wheat seedlings were grown in this series of solutions from March 3 to March 15. To one set of the 66 cultures only the nutrient salts were added, to the second set 50 ppm. of creatinine were added to each culture. Every three days the solutions were changed and analyzed.

When the two sets of cultures had grown for several days, it was noticeable that the creatinine plants were better developed, having broader leaves and longer and well developed roots. This was more noticeable in some of the fertilizer mixtures than in others.

The total growth made in the 66 cultures of nutrient salts without creatinine, designated as normal cultures, was 166.7 grams as against 181.2 grams in the case of the 66 cultures with 50 ppm. of creatinine. Putting the normal at 100, the latter becomes 109, or an increase of 9 per cent as an average of the 66 cultures. As already mentioned, the effect was much more pronounced in certain fertilizer combinations, especially those containing no nitrates, or those low in nitrates. The effects of creatinine in these cultures will now be considered in detail.

Effect of creatinine on growth in cultures containing no nitrate

Table I gives the growth of two sets of cultures composed of mixtures of phosphate and potash, varying in 10 per cent stages,

TABLE I

SHOWING THE EFFECT OF CREATININE ON GROWTH IN CULTURES CONTAINING NO NITRATES

No.	PPM. OF FERTILIZER INGREDIENT IN CULTURE SOLUTION			GREEN WEIGHT OF CULTURE IN GRAMS	
	P_2O_5	NH_3	K_2O	Without creatinine	With creatinine
1	0	0	80	1.400	1.576
2	8	0	72	1.470	2.200
3	16	0	64	1.950	2.100
4	24	0	56	1.527	2.000
5	32	0	48	1.490	2.200
6	40	0	40	1.558	2.408
7	48	0	32	1.795	2.328
8	56	0	24	1.540	2.400
9	64	0	16	1.444	2.220
10	72	0	8	1.400	2.100
11	80	0	0	1.100	1.150

there being no nitrate in the solutions; the concentration was 80 ppm. of $P_2O_5+K_2O$ in each culture. To one set of cultures was added 50 ppm. of creatinine. In the fifth column are given the green weights of the cultures without creatinine, and in the last column are given the weights of the cultures with creatinine. It is apparent from these figures that the creatinine has caused a

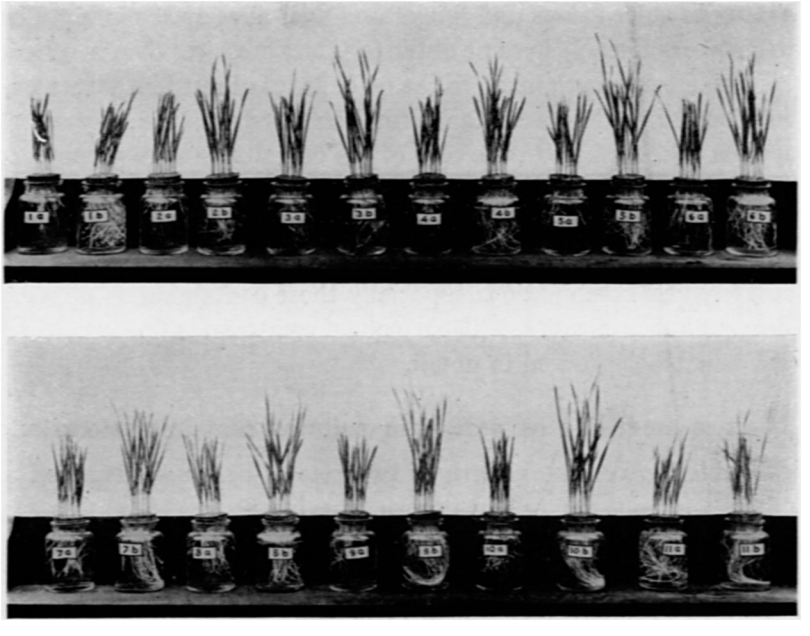

FIG. 1.—Wheat plants growing in culture solutions containing various proportions of potash and phosphate (with no nitrates) without (*a*) and with (*b*) creatinine.

considerable increase in growth. This is true in each of the 11 cultures. The total growth of the eleven cultures, without creatinine, was 16.674 grams against 22.682 grams for the cultures with creatinine. This is an increase of 36 per cent in the creatinine cultures.

The effect of creatinine in cultures with no nitrogen are shown in the plants in fig. 1. Cultures marked with the same number, for instance 1*a* and 1*b*, have similar fertilizer ratios. The cultures marked *a* have no creatinine, the numbers with the letter *b* have

50 ppm. of creatinine. As shown in the photograph, the plants in each culture containing creatinine, regardless of the proportion of potash and phosphate, is larger than the plants grown in a similar solution without the creatinine. The increased growth is noticeable in the roots as well as the tops. The tops in each case are broader and taller, the roots are larger and better branched.

Effect of creatinine in cultures containing 8 ppm. NH_3 as nitrate

Since creatinine was very beneficial in cultures containing no nitrate, it is interesting to observe its effect in cultures which contain a small amount of nitrate. Table II gives the result of the

TABLE II

SHOWING THE EFFECT OF CREATININE ON GROWTH IN CULTURE SOLUTIONS COMPOSED OF FERTILIZER MIXTURES, CONTAINING 8 PPM. OF NH_3 AS NITRATE

No.	PPM. OF FERTILIZER INGREDIENTS IN CULTURE SOLUTION			GREEN WEIGHT OF CULTURE IN GRAMS	
	P_2O_5	NH_3	K_2O	Without creatinine	With creatinine
1.....	0	8	72	1.820	2.190
2.....	8	8	64	2.470	3.100
3.....	16	8	56	2.748	3.250
4.....	24	8	48	2.907	3.420
5.....	32	8	40	2.670	2.450
6.....	40	8	32	2.928	3.258
7.....	48	8	24	2.526	3.340
8.....	56	8	16	2.600	3.000
9.....	64	8	8	2.048	2.359
10.....	72	8	0	1.354	1.750

effect of creatinine on growth in culture solutions composed of 8 ppm. of NH_3 as nitrate, and varying amounts of phosphate and potash, the total concentration of each solution being 80 ppm. of $P_2O_5+NH_3+K_2O$. By comparing the figures it is seen that the growth with creatinine, given in the last column, is larger than the growth without creatinine, given in the fifth column. The difference, however, is not nearly so large as in solutions containing no nitrate, presented in table I. The total green weight of the cultures composed of fertilizer mixtures containing 8 ppm. of nitrogen without creatinine was 24.071 grams against 28.117 grams in the cul-

tures with creatinine, an increase of 17 per cent. In the cultures with no nitrate creatinine produced an increase of 36 per cent.

Effect of creatinine in cultures with larger amounts of nitrate

It has been shown that creatinine was very beneficial in cultures which contained no nitrates. In a group of cultures, composed of mixtures of phosphate and potash in different proportions, creatinine increased the growth 36 per cent. It has also been pointed out that the beneficial effect of creatinine was not so great in cultures containing a small amount of nitrate. In a second group of cultures, composed of mixtures of potash, phosphate, and 8 ppm. of NH_3 as nitrate, creatinine increased the growth only 17 per cent.

In table III are given the results of growth in cultures with and without creatinine, composed of mixtures of phosphate, potash, and nitrogen having 16 ppm. NH_3 as nitrate. The green weights of the creatinine cultures given in the last column of the table are slightly larger than the normal cultures, as shown in the fifth column. The total green weight of the cultures without creatinine was 25.516 grams against 27.573 grams for the cultures with creatinine, an increase of 8 per cent.

TABLE III

SHOWING THE EFFECT OF CREATININE IN CULTURES CONTAINING 16 PPM. OF NH_3 AS NITRATE

No.	PPM. OF FERTILIZER INGREDIENTS IN CULTURE SOLUTION			GREEN WEIGHT OF CULTURES IN GRAMS	
	P_2O_5	NH_3	K_2O	Without creatinine	With creatinine
1	0	16	64	2.200	2.570
2	8	16	56	3.200	3.720
3	16	16	48	3.500	3.500
4	24	16	40	3.097	3.702
5	32	16	32	3.250	3.250
6	40	16	24	3.228	3.300
7	48	16	16	2.975	3.240
8	56	16	8	2.626	2.551
9	64	16	0	1.440	1.740

In other cultures composed of the three fertilizer ingredients P_2O_5, NH_3, and K_2O, but containing 24 ppm. of NH_3 as nitrate, creatinine increased growth only 2 per cent. Its effect in cultures

composed of fertilizer mixtures having more than 24 ppm. of nitrate was uncertain; in some cases there was a slight increase in growth and in others there was a slight decrease, that is, the growth with these higher amounts of nitrate in the solution was practically the same in the normal and creatinine cultures.

Before discussing further the effect of creatinine, it will be necessary to recall the effect which nitrates have on the growth of plants in mixtures of the other two fertilizer ingredients potash and phosphate. In work previously published,[5] it was shown that the better growth occurred in the normal cultures when the three fertilizer elements P_2O_5, NH_3, and K_2O were present. It was best in mixtures which contained approximately equal amounts of NH_3 and K_2O and a small amount of P_2O_5 (about 16 ppm.). The growth in the cultures containing the three constituents was much greater than in the cultures containing only two constituents. This was especially marked when nitrogen was not in the composition. In illustration of this, the average growth of a number of cultures, composed of mixtures of phosphate and potash in amounts of 80 ppm. of $P_2O_5+K_2O$, was 1.000 gram against 3.155 grams as the average growth of cultures composed of mixtures of these two ingredients, with an addition of only 8 ppm. of NH_3 as nitrate, the total concentration of nutrients being the same. In a second experiment conducted in a similar manner, but at a later date, the average growth of the cultures, composed of mixtures of phosphate and potash, was 0.878 gram, and the average growth of cultures, in mixtures of the three ingredients, containing 8 ppm. of NH_3 as nitrate, was 2.107 grams.

In the present experiment the growth in the normal cultures composed of varying proportions of phosphate and potash, compared with the growth in mixtures of these two ingredients, with 8 ppm. of NH_3 as nitrate added, is given in table IV. By a close examination of the figures in this table, it is seen that the growth in the mixtures of phosphate and potash is smaller than in cultures composed of mixtures of the three ingredients, though containing

[5] SCHREINER, O., and SKINNER, J. J., Ratio of phosphate, nitrate, and potassium on absorption and growth. BOT. GAZ. **50**: 1. 1910. Some effects of a harmful organic soil constituent. Bull. 70, Bureau of Soils, U. S. Dept. Agric. 1910.

but 8 ppm. of NH_3. The average growth of the cultures without nitrogen is 1.516 grams against 2.407 grams with 8 ppm. of NH_3 in the fertilizer mixture. Putting the growth of the cultures without nitrogen at 100, the relative growth of the cultures with nitrogen becomes 159, or an increase of 59 per cent.

TABLE IV

SHOWING THE GROWTH OF CULTURES, COMPOSED OF FERTILIZER MIXTURES CONTAINING NO NITRATE, AND 8 PPM. OF NH_3 AS NITRATE, WITHOUT AND WITH CREATININE

No.	PPM. OF FERTILIZER INGREDIENTS IN CULTURE SOLUTION			GREEN WEIGHT OF CULTURES IN GRAMS			
				Without creatinine		With creatinine	
	P_2O_5	NH_3	K_2O	No nitrate	8 ppm. NH_3	No nitrate	8 ppm. NH_3
1.....	0	0	80	1.400	.....	1.576	.....
2.....	0	8	72	.....	1.820	.....	2.190
3.....	8	0	72	1.470	.....	2.200	.....
4.....	8	8	64	.....	2.470	.....	3.100
5.....	16	0	64	1.950	.....	2.100	.....
6.....	16	8	56	.....	2.748	.....	3.250
7.....	24	0	56	1.527	.....	2.000	.....
8.....	24	8	48	.....	2.907	.....	3.420
9.....	32	0	48	1.490	.....	2.200	.....
10.....	32	8	40	.....	2.670	.....	2.450
11.....	40	0	40	1.558	.....	2.408	.....
12.....	40	8	32	.....	2.928	.....	3.258
13.....	48	0	32	1.795	.....	2.328	.....
14.....	48	8	24	.....	2.526	.....	3.340
15.....	56	0	24	1.540	.....	2.400	.....
16.....	56	8	16	.....	2.600	.....	3.000
17.....	64	0	16	1.444	.....	2.220	.....
18.....	64	8	8	.....	2.048	.....	2.359
19.....	72	0	8	1.400	.....	2.100	.....
20.....	72	8	0	.....	1.354	.....	1.750
21.....	80	0	0	1.100	.....	1.150	.....

With 50 ppm. of creatinine in the solution, the cultures containing no nitrogen produced better growth than the corresponding cultures without creatinine, as shown in the last two columns of table IV. The difference between the last two columns is not as marked in the creatinine set as in the corresponding columns for the normal set. The average growth of the creatinine cultures without nitrate is 2.062 grams against 2.812 grams for the cultures having 8 ppm. of NH_3 as nitrate in the fertilizer mixture. If the growth of the cultures without nitrate is put at 100, the growth with 8 ppm.

of NH_3 in the fertilizer mixture becomes 136, or an increase of only 36 per cent. In other words, in the absence of creatinine from the cultures, the nitrate (8 ppm.) caused an average increase of 59 per cent in the various cultures; in the presence of the creatinine (50 ppm.) the nitrate (8 ppm.) caused an average increase of only 36 per cent. It appears, therefore, that plants supplied with creatinine do not respond so markedly to added nitrate, thus seeming to indicate that the plant can utilize this nitrogenous compound for plant syntheses.

Effect of creatinine on absorption of fertilizer salts

The foregoing discussion has shown clearly the influence of creatinine on growth and its effect in cultures containing no nitrates. There remains to be discussed the effect of the creatinine on the removal of nutrients from the solution during the growth of the plant.

Mention has been made already of the fact that the concentration differences produced by the growth of the plants in the various cultures were determined by making an analysis for nitrates at the termination of every three-day change, and of the phosphates and potassium on a composite of the solutions from the four changes. It is thus possible to compare the results obtained under the so-called normal conditions without the creatinine and under the conditions where 50 ppm. of creatinine were present in the solution.

The sum total of P_2O_5, NH_3, and K_2O removed from solution by the growing plants in the cultures containing all three of these constituents was 1684 milligrams under the normal conditions, and 1584 milligrams in the creatinine set. The figures show the total of plant nutrients to be slightly less in the creatinine set, although the green weight in this set was 9 per cent greater than in the normal set. The examination of the results for the three constituents separately as given below shows that the phosphate and potash were slightly greater than normal, as is demanded by the larger growth, whereas the nitrate is considerably less than in the normal set.

Phosphate.—The amount of phosphate stated as P_2O_5 removed from the total number of solutions during the experiment was 364 milligrams for the normal cultures and 383 milligrams for the cul-

tures containing creatinine, a difference of 19 milligrams in favor of the creatinine cultures.

Potassium.—The amount of potash stated as K_2O removed by the plants in the total number of cultures was 760 milligrams in the case of the normal cultures and 778 milligrams for the cultures with creatinine. As with the phosphate, the creatinine cultures removed a little more potash than the normal cultures, there being a difference of 18 milligrams in favor of the creatinine set.

Nitrogen.—The total amount of nitrogen stated as NH_3 removed from the total number of solutions during the course of the experiment was 560 milligrams for the normal cultures and 423 milligrams for the creatinine cultures. The creatinine cultures though making a larger growth used 137 milligrams less nitrate.

Effect of creatine on growth

Creatine is closely related chemically to creatinine, the latter being the anhydride of creatine. Both probably occur in soils, manures, and green crops, a discussion of which is given in the two other papers referred to. Experiments in nutrient cultures with creatine have been conducted similar to those with creatinine.

The plants grew from April 22 to May 4. After the plants had grown for several days, it was apparent that the effect of creatine was very similar to that of creatinine. The leaves were broader, and further developed than those of the normal culture. The roots were longer and better branched. The plants growing in cultures with creatine, which contained phosphate and potash but no nitrate, were a great deal larger than similar cultures without creatine. Like the creatinine, when small amounts of nitrate were in the fertilizer mixture, the beneficial effect of creatine was not so marked, and in the presence of larger amounts of nitrate creatine had no additional effects.

The total green weight of 66 cultures containing the fertilizer salts only, that is the normal set, was 174.4 grams, against 186.8 grams for the 66 cultures containing 50 ppm. of creatine in addition to the fertilizer salts. This is an increase for the creatine cultures of 8 per cent over the normal cultures.

Table V shows the effect of creatine on growth in a number of

cultures containing varying amounts of phosphate and potash, but no nitrates, the amount of total fertilizer ingredient in each culture being 80 ppm. By an examination of the table it is apparent that the growth of each of the creatine cultures given in the last column is considerably larger than the growth of the cultures without creatine given in the fifth column. The total green weight of the cultures without creatine was 16.2 grams against 23.3 grams for the cultures with creatine, an increase of 44 per cent.

TABLE V

SHOWING THE EFFECT OF CREATINE ON GROWTH IN CULTURES CONTAINING NO NITRATE

No.	PPM. OF FERTILIZER INGREDIENT IN CULTURE SOLUTION			GREEN WEIGHT OF CULTURES IN GRAMS	
	P_2O_5	NH_3	K_2O	Without creatine	With creatine
1.....	0	0	80	1.329	1.709
2.....	8	0	72	1.420	1.948
3.....	16	0	64	1.558	2.130
4.....	24	0	56	1.579	2.370
5.....	32	0	48	1.528	2.470
6.....	40	0	40	1.500	2.400
7.....	48	0	32	1.670	2.270
8.....	56	0	24	1.628	2.420
9.....	64	0	16	1.600	2.450
10.....	72	0	8	1.428	2.070
11.....	80	0	0	0.978	1.090

In table VI are given the green weights of plants grown in cultures with and without creatine, containing 8 ppm. of NH_3 as nitrate and varying amounts of P_2O_5 and K_2O, the total constituents being 80 ppm. of $P_2O_5+NH_3+K_2O$. These figures show that the creatine cultures given in the last column are somewhat larger than the cultures without creatine given in the fifth column, but the difference is not nearly so large as in the cultures containing no nitrate given in table V. The total growth of the cultures without creatine was 26.4 grams against 29.4 grams for the cultures with creatine, an increase of only 11 per cent in favor of the creatine cultures. There was a difference of 44 per cent in favor of the creatine cultures in the case of the solution which contained no nitrate.

The growth in the cultures which contained varying amounts of phosphate and potash and 16 ppm. of nitrate was only 3 per cent greater with than without creatine. In solutions containing 24 ppm. of nitrate the increased growth with creatine was 6 per cent, and in solutions containing 32 ppm. nitrate the increased growth 4 per cent. In solutions containing higher amounts of nitrate the creatine had no additional effect. Thus it appears that the effect of creatine in replacing the effect of nitrate in producing growth is very similar to that of creatinine.

TABLE VI

SHOWING THE EFFECT OF CREATINE ON GROWTH IN CULTURES CONTAINING 8 PPM. OF NH_3 AS NITRATE

No.	PPM. OF FERTILIZER INGREDIENT IN CULTURE SOLUTION			GREEN WEIGHT OF CULTURES IN GRAMS	
	P_2O_5	NH_3	K_2O	Without creatine	With creatine
1.....	0	8	72	2.299	2.459
2.....	8	8	64	2.940	3.200
3.....	16	8	56	2.700	3.350
4.....	24	8	48	2.920	3.400
5.....	32	8	40	3.050	3.070
6.....	40	8	32	3.150	3.309
7.....	48	8	24	3.220	3.350
8.....	56	8	16	2.500	2.854
9.....	64	8	8	2.222	2.800
10.....	72	8	0	1.400	1.600

It is also interesting to note the effect of creatine on the removal of salts by the plants and the similarity between the action of creatine and creatinine in this respect. It will be remembered that in the creatinine cultures the removal of phosphate and potash was slightly greater in the creatinine cultures than the normal cultures, but a great deal less nitrate disappeared from solution in the creatinine than in the normal cultures.

In the creatine experiments the removal of total P_2O_5, NH_3, and K_2O by plants in the normal cultures was 1978.3 milligrams, against 1854.5 milligrams for the creatine cultures. The normal cultures removed 471.0 milligrams of P_2O_5 and the creatine cultures 474.4 milligrams. In the case of potash the normal cultures

removed 769.4 milligrams of K_2O against 767.4 milligrams for the creatine cultures. The removal of both phosphate and potash was practically the same in the normal and creatine cultures. The disappearance of nitrate was much less in the creatine than in the normal cultures. The normal cultures removed 737.7 milligrams against 612.7 milligrams for the creatine cultures, a difference of 125 milligrams.

The influence of the creatine in regard to the removal of P_2O_5, NH_3, and K_2O is very similar to that shown by creatinine, and it again appears that this substance as well as the creatinine can replace nitrates in its effect on plant growth.

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